Biologia Futura (2022) 73:301–308
https://doi.org/10.1007/s42977-022-00129-0

ORIGINAL PAPER

Ion contents, physiological characteristics and growth of *Carum copticum* as influenced by salinity and alkalinity stresses

Zahra Dehghan-Harati[1] · Batool Mahdavi[1] · Seyedeh-Elahe Hashemi[1]

Received: 1 December 2020 / Accepted: 28 July 2022 / Published online: 5 October 2022

Abstract
A controlled experiment was conducted to investigate the effect of salinity and alkalinity stresses on the growth and physiological characteristics of *Carum copticum* L. The treatments included four salinity levels: 0, 50, 100 and 150 mM NaCl, and four alkalinity levels 0, 20, 40 and 60 mM $NaHCO_3$. The results indicated that root dry weight and magnesium concentration were decreased and catalase and peroxidase activity, proline, malondialdehyde, Na^+ and Ca^{2+} concentration were increased in plants simultaneously subjected to both salinity and alkalinity stresses. In all traits, the highest salinity and alkalinity levels had the most negative and significant effects. In general, our findings revealed that alkalinity and salinity stresses considerably decreased ajwain growth through adverse impact on physiological characteristics such as ion concentration and activity of antioxidant enzymes. These effects were greater when the two salinity and alkalinity stresses were simultaneously applied. Ajwain plant tolerated a part of the stresses via osmotic adjustment mechanism was assessed by proline, malondialdehyde and total carbohydrate.

Keywords Alkali salt · Antioxidant enzymes · Malondialdehyde · Osmotic adjustment

Introduction

Environmental stresses such as high and low temperatures, drought, alkalinity and salinity affect plants morphology and physiology and damage their potential growth and yield (Aydin et al. 2011; Azooz and Ahmad 2016). The salinity stress can impact photosynthesis, nitrogen and carbon metabolisms in plants and cause distractions in ion uptake by plants. Salt stress also can decrease some nutritional elements and affect plant nutrition. These physiological variations can lead to growth retardation and reduced function in plants (Munns and Tester 2008). Plants adapt to saline conditions by the accumulation of some organic salts and synthesis of osmolytes such as proline, sugars, multifunctional group alcohols and amino acids (Hoque et al. 2007). In higher salinity levels, this osmotic regulation protects the cellular structures and reduces the oxidative damages of reactive oxygen species (ROS) (Munns and Tester 2008).

The alkalinity stress happens when a soil contains high content of alkali salts (Na_2CO_3 and $NaHCO_3$) and can impose more harmful damages compared to the neutral salts (NaCl and Na_2SO_4). Bicarbonates (HCO_3^-) and carbonates (CO_3^{2-}) are the primary reasons for the alkalinity; however, hydroxides, borate, ammonium, organic bases, phosphates and silicates are also suggested as additional factors (Ahmad et al. 2014). So, if the soil contains more HCO_3^- or CO_3^{2-}, pH will increase and the plants will be influenced by both salinity and alkalinity stresses. The bicarbonate ion can significantly inhibit growth in species that are sensitive to pH. High soil pH can lead to the reducing root physiological function and destructing root cell structure by insolubilization and sedimentation of metal and phosphorus ions (Li et al. 2009). Furthermore, alkalinity stress leads to disruption of hydrogen bonds of cellulose and inhibition of cell development by damaging the cell wall and consequently leads to growth retardation. Availability of the nutrients such as zinc, manganese and iron significantly decreases in the alkali conditions (Bugbee 2003), and phosphorus with calcium and magnesium creates metal complexes and they get unreachable for the plants (Nikolic and Kastori 2000).

Ajwain (*Carum copticum*) as an herbaceous plant has highly branched shoots and leaves with large cuts

✉ Batool Mahdavi
b.mahdavi@vru.ac.ir

[1] Present Address: Department of Genetics and Plant Production, Agriculture College, Vali-e-Asr University of Rafsanajn, Rafsanjan, Iran

and relatively round fruits. The seed of ajwain is thymol enriched and contains a high amount of cymene, alpha and beta-pinene. The main components of ajwain essential oil are thymol, γ-terpinene and para cymene, and the smell of seeds is due to thymol (Raeisi et al. 2016). Most researches on plants physiological response to salinity mainly focus on reactions of plants to salt stress caused by NaCl; however, there is low information about plants response to salt stress caused by alkali salt. This lack of information has been more severe in medicinal plants. Therefore, this research was conducted to examine the ion concentration, physiological characteristics and growth of ajwain as the influence of salinity and alkalinity stresses.

Materials and methods

This research was conducted at the Research Greenhouse of College of Agriculture, Vali-e-Asr University of Rafsanjan, Iran.

Ten uniform ajwain seeds were planted in the pots with 20 cm diameter and 30 cm height, which were filled with perlite and cocopeat (1:1). The plants were thinned to 5 pot^{-1} after seedlings emergence. The treatments included four salinity levels: 0, 50, 100 and 150 mM NaCl, and four alkalinity levels: 0, 20, 40 and 60 mM $NaHCO_3$. The salinity and alkalinity stresses treatments were applied after the 4-leaf stage through half-strength Hoagland's solution. The half-strength Hoagland's nutrient solution included 0.75 mM $MgSO_4$, 0.5 mM KH_2PO_4, 1.25 mM KNO_3, 1.5 mM $Ca(NO_3)_2$, 50 µM KCl, 50 µM H_3BO_3, 10 µM $MnSO_4$, 2 µM $ZnSO_4$, 1.5 µM $CuSO_4$, 0.075 µM $(NH_4)_6Mo_7O_{24}$, 10 µM Fe-EDTA with 50% phosphorous. The concentration of the desired salt in the Hoagland nutrient solution was prepared by applying sodium chloride and sodium bicarbonate. Control plants (non-stress treatment) were only irrigated with half-strength Hoagland's solution. The minimum and maximum temperature in the greenhouse was 14 °C and 28 °C and relative humidity was 55–65%. The ajwain plants received 14 h daily light from a combination of tungsten and fluorescent lamps.

At 52 days after treatment applying, the 66-day-old seedling was subjected to the sampling. The distance between the crown and the highest leaf was measured as shoot length, and the distance between the crown to the tip of the longest root was determined as root length. The aerial and belowground parts of the plant were separated and were washed with distilled water to measure root and shoot weight. The samples were dried in oven at 70 °C for 48 h, and afterward the dried weights of the above- and belowground samples were considered as shoot and root dry weight. Relative water content (RWC) was measured using fresh weight (FW), dry weight (DW) and turgid weight (TW) in leaves according the following formula:

$$\mathrm{RWC} = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100 \quad (1)$$

The content of free proline and soluble carbohydrates in leaves were calculated using Bates et al. (1973) and Irigoyen et al. (1992) protocols, respectively. Peroxidation of membrane lipids was determined by the assessing of malondialdehyde (MDA) as the end product of peroxidation of membrane lipids, which was measured based on De Vos et al. (1991) protocol. The method introduced by Cakmak and Marschner (1988), Pandolfini et al. (1992) and Giannopolitis and Ries (1977) was, respectively, followed to measure the activity of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzymes. The concentration of ions was measured by the digestion of burning dry plant samples in an oven with 550 centigrade for 6 h. Potassium (K^+) and sodium (Na^+) concentrations were measured by a flame photometer (Model PFP7, Germany). The titration method was used to assay calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations.

The experiments were performed in a randomized complete block design arranged as a factorial with three replications. After the normality test, the collected data were subjected to analysis of variance (ANOVA) and the means were compared using (LSD 0.01). All statistical analysis was done by SAS software.

Results

The results of variance analysis showed that the effect of salinity and alkalinity and their interaction effects were significant on root dry weight. In contrast, shoot dry weight was significantly affected by salinity and alkalinity (Table 1). Salinity and alkalinity interaction had an additive effect on root dry weight, so that increasing alkalinity to 60 mM was associated with reduced root dry weight by 53.6% than no-alkaline in non-saline conditions (Fig. 1). While in saline conditions, the different alkalinity levels had no significant effect on root dry weight. With increasing salinity and alkalinity, the dry weight of the shoot decreased (Tables 2 and 3). By applying 150 mM salinity and 60 mM alkalinity stresses, shoot dry weight was decreased by 36.1% and 10.8% compared to no-stress conditions, respectively (Tables 2 and 3).

The results of data analysis showed that the shoot and root length was only influenced by salinity (Table 1). With increasing salt stress levels, shoot and root length were significantly decreased (Tables 2 and 3). In 150 mM salinity, shoot and root length were decreased by 35 and 24%, respectively, compared to non-saline conditions.

Table 1 The analysis of variance (ANOVA) for some characteristics of ajwain under salinity and alkalinity

Source of variance	D.F†	F value								
		RDW	SDW	SL	RL	RWC	MDA	SP	SC	TSP
Block (R)	2, 30	1.20 ^{ns}	0.80 ^{ns}	1.44 ^{ns}	0.42 ^{ns}	4.95 *	1.75 ^{ns}	2.01 ^{ns}	0.82 ^{ns}	1.85 ^{ns}
Salinity (S)	3, 30	11.02 **	60.63 **	10.69 **	14.62 **	10.94 **	16.05 **	14.53 **	20.25 **	10.01 **
Alkalinity (A)	3, 30	20.06 **	4.63 *	0.40 ^{ns}	1.87 ^{ns}	0.35 ^{ns}	14.78 **	17.01 **	8.02 **	7.95 **
S × A	9, 30	12.05 **	2.10 ^{ns}	0.37 ^{ns}	0.099 ^{ns}	0.19 ^{ns}	27.51 **	10.25 **	1.23 ^{ns}	0.46 ^{ns}
CV (%)		11.93	8.63	13.59	10.67	5.74	6.27	7.96	11.87	12.49

ns, * and **: nonsignificant, significant at 5% and 1% of probability levels, respectively

†: The first and second DFs belong to each SOV and error, respectively

D.F degree of freedom; *CV* coefficient of variation, *RDW* root dry weight, *SDW* shoot dry weight, *SL* shoot length, *RL* root length, *RWC* relative water content, *MDA* malondialdehyde *SP* soluble proline, *SC* soluble carbohydrates, *TSP* total soluble protein,

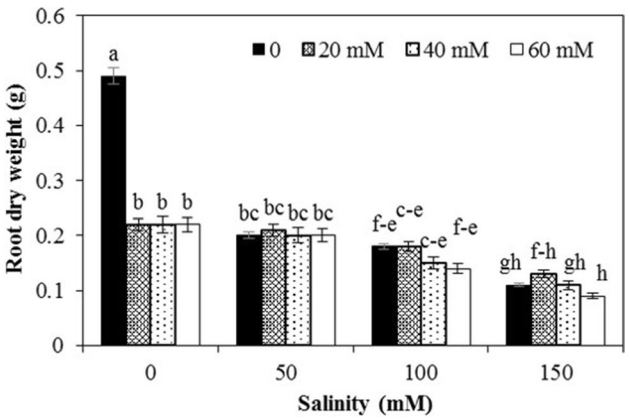

Fig. 1 The interaction of different salinity and alkalinity levels on root dry weight. Means with at least a similar letter are not significantly differed based on the LSD test (0.05). Values are means ± SE of three replicates

The relative water content (RWC) was only influenced by salinity (Table 1), as the RWC was decreased with increasing salinity level (Table 2). The plants grown in non-saline conditions had the highest RWC; however, it was not significantly different from 50 mM salinity. In 150 mM salinity stress, an 8.72% reduction in RWC was observed compared to non-saline conditions. There was no significant difference between 150 and 100 mM salinity (Table 2).

The effect of salinity, alkalinity and their interaction was significant on the content of malondialdehyde (MDA) and proline. Soluble carbohydrates and total proteins only were affected by salinity and alkalinity effects (Table 1). Salinity and alkalinity interaction led to a significant increase in MDA content (Fig. 2). In non-saline conditions, increased alkalinity resulted in the enhanced MDA content. In all salinity conditions, different alkalinity levels caused an increase in MDA content in compared to non-alkaline conditions (Fig. 2).

Increased salinity and alkalinity led to an increase in proline content. In non-saline conditions and 50 mM salinity, different alkalinity levels had significantly equal proline content. While 40 and 60 mM alkalinity stress was associated with enhanced proline content in 100 and 150 mM salinity (Fig. 2). The content of total carbohydrates was increased by increasing salinity stresses levels (Table 2). The highest total carbohydrates content was observed in 150 mM salinity that was 35.1% higher than non-saline conditions. Also, with increasing alkalinity to 40 and 60 mM, the carbohydrates content was increased (Table 3). Total soluble proteins were reduced with increasing salinity and alkalinity stresses (Tables 2 and 3), in order to protein content was 22.2% less in 150 mM salinity than non-saline conditions

Table 2 The effect of salinity on some growth and biochemical traits of ajwain

Salinity (mM)	SDW (g)	SL (cm)	RL (cm)	RWC (%)	SC (mg g^{-1})	TSP (mg g^{-1})	K^+ (%)	SOD (mg mg^{-1} protein)
0	1.03 ± 0.82a	36.58 ± 2.61a	19.00 ± 1.77a	62.06 ± 2.55a	0.083 ± 0.006c	0.812 ± 0.041a	3.45 ± 0.47a	32.68 ± 6.21b
50	0.92 ± 0.71b	33.66 ± 2.55b	17.33 ± 1.56ab	59.60 ± 2.45ab	0.100 ± 0.009b	0.708 ± 0.033b	2.88 ± 0.33b	42.18 ± 5.34a
100	0.80 ± 0.63c	31.16 ± 2.34b	15.91 ± 1.62b	57.03 ± 2.35bc	0.109 ± 0.008ab	0.676 ± 0.034bc	2.66 ± 0.31bc	45.20 ± 4.55a
150	0.66 ± 0.70d	27.62 ± 2.13c	14.00 ± 1.67c	54.65 ± 1.99c	0.112 ± 0.007a	0.632 ± 0.035c	2.38 ± 0.32c	46.60 ± 5.01a

SDW shoot dry weight, *SL* shoot length, *RL* root length, *RWC* relative water content, *SC* soluble carbohydrates, *TSP* total soluble protein, K^+ potassium concentration, *SOD* superoxide dismutase. In each column, means with at least a similar letter are not significantly differed based on the LSD test (0.05). Values are means ± SE of three replicates

and was 20.0% less in 60 mM alkalinity than no alkalinity stress.

Potassium (K^+) concentration was only significantly influenced by salinity, while salinity and alkalinity and their interaction effects had a significant effect on sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations in ajwain shoot (Table 4). Shoot K^+ concentration was significantly decreased with increasing salinity level (Table 2); so, the lowest K^+ concentration was obtained in 150 mM salinity treatment and the plants grown in non-saline conditions had the highest K^+ concentration. With increasing salinity and alkalinity levels, Na^+ concentration was significantly increased (Fig. 3). Under non-saline and all salinity conditions, 60 mM alkalinity considerably increased Na^+ concentration compared to the no-stressed plants.

The calcium concentration was increased by increasing salinity and alkalinity stresses (Fig. 3). In non-saline, 50 and 100 mM salinity, alkalinity stress had no significant effect on Ca^{2+}. In contrast, in 150 mM salinity, 40 and 60 mM alkalinity treatments resulted in a significant increase in Ca^{2+} concentration compared to no alkalinity stress conditions (Fig. 3). The increase of salinity and alkalinity stresses

Table 3 The effect of alkalinity on some growth and biochemical traits of ajwain

Alkalinity level (mM)	SDW (g)	SC (mg g^{-1})	TSP (mg g^{-1})	SOD (mg mg^{-1} protein)
0	0.89 ± 0.031a	0.091 ± 0.011c	0.772 ± 0.033a	26.30 ± 5.34b
20	0.88 ± 0.036a	0.098 ± 0.010bc	0.743 ± 0.039ab	45.61 ± 6.67a
40	0.87 ± 0.030a	0.104 ± 0.008ab	0.696 ± 0.041b	46.50 ± 7.21a
60	0.79 ± 0.042b	0.111 ± 0.009a	0.616 ± 0.050c	48.24 ± 5.69a

SDW shoot dry weight, *SC* soluble carbohydrates, *TSP* total soluble protein, *SOD* superoxide dismutase. In each column, means with at least a similar letter are not significantly differed based on the LSD test (0.05). Values are means ± SE of three replicates

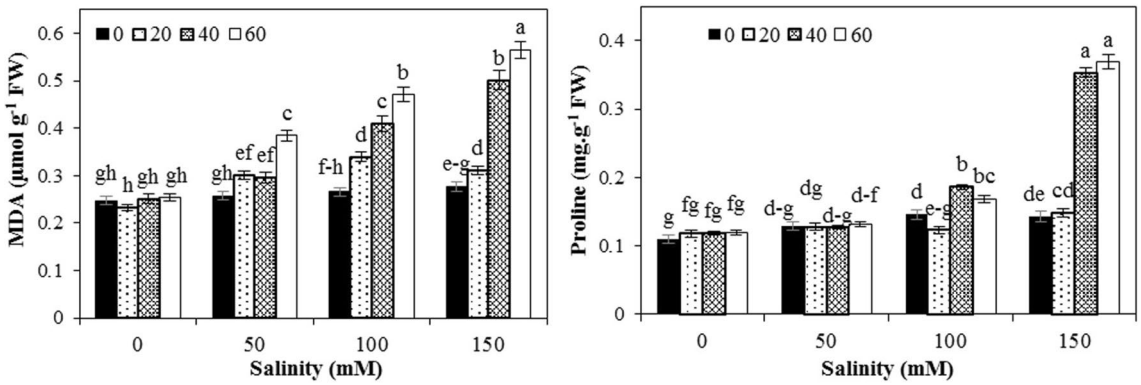

Fig. 2 The interaction of different salinity and alkalinity levels on MDA and proline content. In each figure, means with at least a similar letter are not significantly differed based on the LSD test (0.05). Values are means ± SE of three replicates

Table 4 The analysis of variance (ANOVA) for ion concentration and antioxidant enzymes of ajwain under salinity and alkalinity

Source of variance	D.F†	F value						
		K^+	Na^+	Ca^{2+}	Mg^{2+}	CAT	POD	SOD
Block (R)	2, 30	1.36 ns	0.18 ns	1.86 ns	1.06 ns	4.35*	1.27 ns	0.38 ns
Salinity (S)	3, 30	24.25**	104.38**	16.63**	8.66**	16.03**	14.52**	16.26**
Alkalinity (A)	3, 30	2.17 ns	11.23**	9.51**	25.06**	14.57**	9.48**	10.43**
S × A	9, 30	1.06 ns	4.38*	5.77**	6.20**	8.53**	4.77**	1.62 ns
CV (%)		6.10	10.22	10.48	8.37	12.10	12.13	12.99

ns, * and **: nonsignificant, significant at 5% and 1% of probability levels, respectively

†: The first and second DFs belong to each SOV and error, respectively

D.F. degree of freedom; *CV* coefficient of variation, K^+ potassium concentration, Na^+ sodium concentration, Ca^{2+} calcium concentration, Mg^{2+} magnesium concentration, *CAT* catalase, *POD*: peroxidase, *SOD* superoxide dismutase

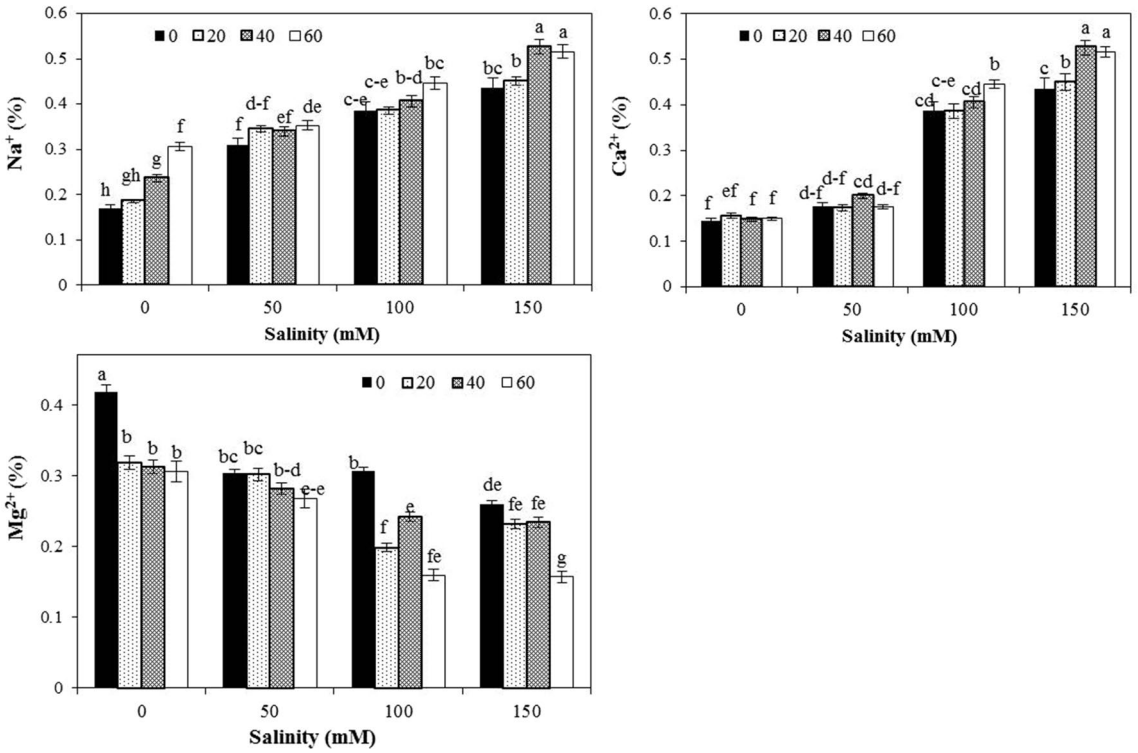

Fig. 3 The interaction of different salinity and alkalinity levels on sodium, calcium and magnesium concentrations in ajwain shoot. In each figure, means with at least a similar letter are not significantly differed based on the LSD test (0.05). Values are means ± SE of three replicates

decreased Mg^{2+} concentration. Increasing alkalinity had no significant effect on Mg^{2+} in 50 mM salinity, whereas 60 mM alkalinity remarkably reduced Mg^{2+} concentration in 0, 100 and 150 mM salinity conditions (Fig. 3).

The effect of salinity and alkalinity were significant on the activity of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) and their interaction effect was only significant on the activity of CAT and POD (Table 4). The increase in salinity and alkalinity levels led to the increased activity of CAT and POD (Fig. 4). In non-saline and 50 mM salinity, alkalinity stress had no significant effect on CAT activity. However, in 100 and 150 mM salinity levels, all alkalinity levels led to considerably greater CAT activity than non-alkaline stress condition. In the non-saline situations, there was no difference between POD activity in alkalinity treatments (Fig. 4), whereas 60 mM alkalinity increased POD activity in 50 mM salinity. In 100 and 150 mM salinity, 40 and 60 mM alkalinity increased POD

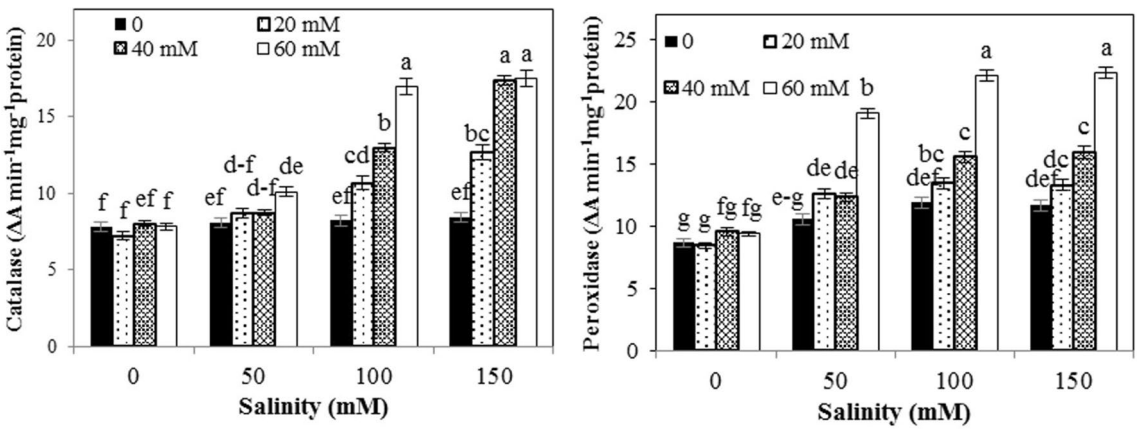

Fig. 4 The interaction of different salinity and alkalinity levels on catalase and peroxidase activity in ajwain shoot. In each figure, means with at least a similar letter are not significantly differed based on the LSD test (0.05). Values are means ± SE of three replicates

activity than without alkalinity stress. Alkalinity and salinity stress in all levels led to enhanced activity of SOD compared to the condition without stress (Tables 2 and 3). The highest activity of SOD was observed in 150 mM salinity stress (42.6% than non-saline treatment) (Table 2) and also in 60 mM alkalinity stress (83.4% than non-alkaline treatment) (Table 3).

Discussion

The result indicated salt stress decreased shoot and root dry weight, and the negative effect of salt stress was greater as salinity was intensified. Our finding agreed with previous research, as Javed et al. (2014) observed that increased salinity level was parallel with reduced growth of shoot and root of rapeseed, due to the low uptake of essential nutrition, ion imbalance and the effect of toxic ions, especially sodium. Pirasteh-Anosheh et al. (2017) reported that shoot and root dry weights decreased in the higher salinity levels in barley. In the current research, alkalinity stress at the highest level was associated with a reduction in shoot dry weight. In alkalinity stress, the effect of high pH in the environment leads to insolubilization and sedimentation of metal ions and phosphorus. It leads to the low physiological function of the root and destruction of the root cell (Li et al. 2009). It is reported that the shoot weight was decreased by increasing sodium bicarbonate also in grapes (Nikolic and Kastori 2000).

Salt and alkaline stresses, depending on their levels, decreased shoot and root length. The variations in shoot and root length are among the most critical signs of plants response to abiotic stresses (Jamil et al. 2006). Erdal and Cakirlar (2014) reported reducing safflower height in response to the increased salinity level. The reduced shoot growth as an influence of salinity can be due to low water uptake, photosynthesis inhibition and reduced carbohydrate synthesis. Furthermore, salinity and alkalinity in plants lead to metabolism disorders and consequently growth retardation in plants. Increased salinity in rapeseed can also affect cell division and shoot and root vertical growth (Bybordi et al. 2010).

In the current study, only salt stress decreased RWC, so the lowest RWC was obtained in 150 mM salt stress. The RWC was also reduced in rice (Lv et al. 2013) and jojoba (Hassan and Ali 2014) plants by increasing salinity stress. The regulation of RWC in salinity conditions is a part of the tolerance process, as both water and minerals determine the turgor pressure. Generally, the plants that reach osmotic balance faster in salinity conditions can adapt to their condition more quickly and can retain desirable state of RWC by better water uptake (Azooz and Ahmad 2016).

Our finding showed that salt stress at all levels increased MDA. The salt stress leads to enhanced production and accumulation of reactive oxygen species (ROS), probably inducing an oxidative stress. It creates protein and lipid oxidations and causes the destruction of membrane structure and the increased MDA (Molassiotis et al. 2006). Malondialdehyde content was also enhanced as affected by alkalinity in saline conditions. In other word, alkalinity had no significant effect on MDA in non-saline conditions. Alkalinity stress has more impact on the production and accumulation of metabolites such as MDA than salinity stress (Guo et al. 2015).

Although both salt and alkaline stresses led to higher proline production, there was some difference in their effects. So that, salt stress at 100 and 150 mM significantly increased proline content at all alkaline levels; but the highest values were only recorded in combination with alkalinity at 40 and 60 mM. The plants enhance organic contents and mineral osmolytes in the cytosol for more salinity and alkalinity tolerance (Chiraz et al. 2012). Production of an osmolyte such as proline is one of the major adaptive mechanisms against salinity stress in plants. It was reported that the proline content was increased in strawberry cultivars by increasing the alkalinity (Ahmad and Sharma 2010). Under salinity stress, the increase of proline content was also observed in *Chloris virgata* (Li et al. 2009). An increased proline content was reported in *Oryza sativa* by a simultaneous increasing salinity and alkalinity levels (Lv et al. 2013), which agree with our results.

Salt stress at all levels and alkalinity stress at 40 and 60 mM significantly increased soluble carbohydrates in the ajwain plant. In stressed plants, the accumulation of soluble carbohydrates can preserve the cell membrane against high salt concentrations and different reactive oxygen species (Ahmad and Sharma 2010). During the stress conditions, plants need to sustain water potential inside the cell to preserve turgor pressure and water uptake for growth, which is created by synthesizing osmotic regulators such as soluble sugars. The carbohydrates are increased in salinity stress conditions in some safflower species (Javed et al. 2014) and in salinity and alkalinity stresses in *Avena sativa* (Guo et al. 2011a). Total soluble proteins content was decreased as salinity and alkalinity stresses were imposed. However, this effect was observed in all salt stress and only in 40 and 60 mM alkalinity. Reduction in total soluble proteins content happens due to the protein hydrolysis by proteolytic process under salinity stress (Parida and Das 2005). The reduction of soluble proteins is reported by increasing salinity stress in *Carthamus tinctorius* (Javed et al. 2014) and *Cicer arietinum* (Keshavkant et al. 2012).

Only salt stress significantly reduced K^+ concentration; however, both salt and alkaline stresses in an additive manner increased Na^+ concentration. The plant adaptation to salinity stress is mainly related to the tissue tolerance to accumulated Na^+ and/or Cl^- as well as osmotic stress

(Pirasteh-Anosheh et al. 2017); however, plants, in general, differ greatly in their extent of salinity tolerance (Munns and Tester 2008). Jimenez-Bremont et al. (2006) indicated that higher K^+ and lower Na^+ in plant tissue could be considered as salinity tolerance. According to the antagonistic relationship between K^+ and Na^+, salinity was associated with reduced K^+ in shoot and root (Karmoker et al. 2008). In agreement with our finding, some researchers reported less K^+ in cotton (Guo et al. 2014) and rice (Lv et al. 2013) grown in salinity. Furthermore, enhanced Na^+ concentration in *Setaria viridis* (Guo et al. 2011b) was also documented in saline and alkaline conditions.

On the other hand, salt stress of more than 50 mM increased Ca^{2+} in all alkaline levels; however, the effect of alkalinity depended on salinity. So, only 60 mM alkalinity in 100 mM salinity and 40 and 60 mM alkalinity in 150 mM salinity enhanced Ca^{2+} concentration. Calcium availability is necessary for membrane stability, signalling pathways and cell wall synthesis (Guo et al. 2014). Salinity impaired the accumulation of K^+ and Ca^{2+} by affecting the transport system of the plasma membrane through K^+ -or Ca^{2+} channels (Munns and Tester 2008); optimum concentrations of these ions are required to maintain the stability and functioning of cell membranes and associated enzymes (Pirasteh-Anosheh et al. 2017). In some cases, it has been shown that the accumulation of Ca^{2+} can reduce the poisonous effects of salt stress; which is due to the increased selective uptake of K^+ (Parida and Das 2005). Calcium is in positive accordance with alkalinity in such a situation, the increased Ca^{2+} concentration leads to the blockade of the SOS system (the signalling pathway system of the cell in stress tolerance) and the damage reduction caused by Na^+ (Guo et al. 2015). Increased Ca^{2+} has been reported by increased salinity and alkalinity stresses in wheat (Guo et al. 2009), *Glycine max* (Ge et al. 2011). Our results showed that salt stress and alkalinity in an almost similar way decreased Mg^{2+}. Their effect closely depended on their levels. Al-Abdoulhadi et al. (2012) reported the reduced Mg^{2+} concentration in shoots of *Phoenix dactylifera*, and Akram et al. (2009) reported the same result in *Helianthus annuus* in response to salinity increase. Alkalinity is associated with the presence of Na^+ in the soil, where Na_2CO_3 or $NaHCO_3$ damage plants by Na^+ toxicity and also by high pH. However, relatively few studies have been undertaken on the effects of alkaline soils on plant growth and productivity (Ahmad et al. 2014).

In general, salt and alkaline stresses enhanced the activity of CAT, POD and SOD; however, these two stresses had significant interaction effects only on CAT and POD. The highest activity of CAT and POD was observed in ajwain plants treated with 150 mM salinity and 60 mM alkalinity. It seems the ajwain plants reacted to the simultaneous salinity and alkalinity stresses by increasing CAT and POD activity. Avoiding the production of ROS during stress is an essential strategy for plants to overcoming stress (De Carvalho 2008). Keshavkant et al. (2012) reported that CAT activity closely related to salinity tolerance in *Cicer arietinum*. Enhanced CAT and POD activity in salinity and alkalinity stresses was reported in *Chenopodium album* L. (Chen et al. 2012). According to the profound role of POD in enzymatic H_2O_2 scavenging and maintenance of cell membrane integrity, the enhanced POD in the plants grown under saline conditions would be expected (Jaleel et al. 2008). Although SOD activity was induced by salt and alkalinity stresses, there is no significant difference between the varying stress levels. The increased SOD enzyme activity in this research is in accordance with the findings in bean (Aydin et al. 2011) and jojoba (Hassan and Ali 2014) under salinity and alkalinity stresses. The alkalinity at the same time severely upsets the mechanism of ion uptake in both populations. Not much work has been conducted on antioxidant studies under alkalinity stress (Ahmad et al. 2014).

Conclusion for future biology

Our findings showed alkalinity and salinity had a negative effect on plant growth by reducing the osmotic potential and consequently less uptake of water and essential elements such as K^+ and Mg^{2+}. Hence, it caused growth inhibition in the root and shoot of the ajwain plants. The adverse effects of alkalinity stress of more than 40 mM were more intensive. Simultaneous salinity and alkalinity stresses with synergistic interaction had more destructive effects on ajwain growth and physiological functions rather than every single stress. The increasing salinity and alkalinity levels also reduced protein contents and enhanced total proline, soluble carbohydrates and activity of CAT, POD and SOD.

Author contributions This manuscript has been written using some of the data from a project, which was conducted by Dr. BM and her students ZD-H and SEH. All of them contributed to conducting as well as drafting.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Ahmad P, Sharma S (2010) Physio-biochemical attributes in two cultivars of mulberry (*Morus alba* L.) under $NaHCO_3$ stress. Int J Plant Prod 4:1735–1743

Ahmad P, Ozturk M, Sharma S, Gucel S (2014) Effect of sodium carbonate-induced salinity–alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (*Morus alba* L.) cultivars. J Plant Interact 9:460–467

Akram MS, Ashraf M, Akram NA (2009) Effectiveness of potassium sulfate in mitigating salt-induced adverse effects on different physio-biochemical attributes in sunflower (*Helianthus annuus* L.). Flora Morphol Distrib Funct Ecol Plants 204:471–483

Al-Abdoulhadi IA, Dinar HA, Ebert G, Buuml C (2012) Influence of salinity stress on photosynthesis and chlorophyll content in date palm (*Phoenix dactylifera* L.) cultivars. Afr J Agric Res 7:3314–3319

Aydin A, Kant C, Turan M (2011) Hydrogel substrate alleviates salt stress with increase antioxidant enzymes activity of bean (*Phaseolus vulgaris* L.) under salinity stress. Afr J Agric Res 6:715–724

Azooz MM, Ahmad P (2016) Plant-environment interaction: responses and approaches to mitigate stress. Wiley, London

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Bugbee B (2003) Nutrient management in recirculating hydroponic culture. S Pac Soil Culture Conf 648:99–112

Bybordi A, Tabatabaei SJ, Ahmedov A (2010) Effect of salinity on the growth and peroxidase and IAA oxidase activities in canola. J Food Agric Environ 8:109–112

Cakmak I, Marschner H (1988) Enhanced superoxide radical production in roots of zinc-deficient plants. J Exp Bot 39:1449–1460

Chen S, Xing J, Lan H (2012) Comparative effects of neutral salt and alkaline salt stress on seed germination, early seedling growth and physiological response of a halophyte species (*Chenopodium glaucum*). Afr J Biotechnol 11:9572–9581

Chiraz CH, Afef HN, Carrayol HA, Habib GM, Houda G (2012) Long-term salt stress responsive growth, carbohydrate metabolism and proline and anti-stress enzymes in *Nicotiana tabaccum*. Afr J Biotechnol 11:8117–8126

De Carvalho MHC (2008) Drought stress and reactive oxygen species. Plant Signal Behav 3:156–165

De Vos C, Schat H, De Waal M, Vooijs R, Ernst W (1991) Increased to copper-induced damage of the root plasma membrane in copper tolerant *Silene cucubalus*. Plant Physiol 82:523–528

Erdal SC, Cakirlar H (2014) Impact of salt stress on photosystem II efficiency and antioxidant enzyme activities of safflower (*Carthamus tinctorius* L.) cultivars. Turk J Biol 38:549–560

Ge Y, Li Y, Lv DK, Bai XJIW, Cai H, Wang AX, Zhu YM (2011) Alkaline-stress response in *Glycine soja* leaf identifies specific transcription factors and ABA-mediated signaling factors. Funct Integr Genomic 11:369–379

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occur Higher Plants Plant Physiol 59:309–314

Guo R, Shi L, Yang Y (2009) Germination, growth, osmotic adjustment and ionic balance of wheat in response to saline and alkaline stresses. Soil Sci Plant Nutr 55:667–669

Guo R, Zhou J, Hao W, Gong D, Yang ST, Zhong X, Gu FX (2011a) Comparison of the effects of saline and alkaline stress on growth, photosynthesis and water-soluble carbohydrate of oat seedling (*Avena sativa* L.). Princ Appl Assess Soil Sci 6:118–128

Guo R, Zhou J, Hao W, Gong D, Zhong X, Gu F, Liu Q, Xia X, Tian J, Li H (2011b) Germination, growth, photosynthesis and ionic balance in *Setaria viridis* seedlings subjected to saline and alkaline stress. Can J Plant Sci 91:1077–1088

Guo R, Zhou J, Hao W, Gu F, Liu Q, Li H, Xia X, Lili M (2014) Germination, growth, chlorophyll fluorescence and ionic balance in linseed seedlings subjected to saline and alkaline stresses. Plant Prod Sci 17:20–31

Guo R, Yang Z, Li F, Yan C, Zhong X, Liu Q, Xia X, Li H, Zhao L (2015) Comparative metabolic responses and adaptive strategies of wheat (*Triticum aestivum*) to salt and alkali stress. BMC Plant Biol 15:1–13

Hassan F, Ali E (2014) Effects of salt stress on growth, antioxidant enzyme activity and some other physiological parameters in jojoba [*Simmondsia chinensis* (link) schneider] plant. Aust J Crop Sci 8:1615

Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007) Exogenous proline mitigates the detrimental effects of salt stress more than the betaine by increasing antioxidant enzyme activities. J Plant Physiol 164:553–561

Irigoyen JJ, Einerich DW, Sanchez-Diaz M (1992) Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol Plant 84:55–60

Jaleel CA, Gopi R, Kishorekumar A, Manivannan P, Sankar B, Panneerselvam R (2008) Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiol Plant 30:287–292

Jamil M, Lee DB, Jung KY, Ashraf M, Lee SC, Rha ES (2006) Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. J Cent Eur Agric 7:273–282

Javed S, Bukhari SA, Ashraf MY, Mahmood S, Iftikhar T (2014) Effect of salinity on growth, biochemical parameters and fatty acid composition in safflower (*Carthamus tinctorius* L) Pak J Bot 46(4):1153–1158

Jimenez-Bremont JF, Becerra-Flora A, Hernandez-Lucero E, Rodriguez-Kessler M, Acosta-Gallegos JA, Ramirez-Pimentel JG (2006) Proline accumulation in two bean cultivars under salt stress and the effect of polyamines and ornithine. Biol Plant 50:763–766

Karmoker JL, Farhana S, Rashid P (2008) Effects of salinity on ion accumulation in maize (*Zea mays* L cv Bari-7) Bangladesh J Bot 37(2): 203–5

Keshavkant S, Padhan J, Parkhey S, Naithani SC (2012) Physiological and antioxidant responses of germinating *Cicer arietinum* seeds to salt stress. Russ J Plant Physiol 59:232–237

Li C, Fang B, Yang C, Shi D, Wang D (2009) Effects of various salt-alkaline mixed stresses on the state of mineral elements in nutrient solutions and the growth of alkali resistant halophyte *Chloris virgata*. J Plant Nutr 32:1137–1147

Lv BS, Li XW, Ma HY, Sun Y, Wei LX, Jiang CJ, Liang ZW (2013) Differences in growth and physiology of rice in response to different saline-alkaline stress factors. Agron J 105(4):1119–1128

Molassiotis A, Tanou G, Diamantidis G, Therios L (2006) Effects of 4-month Fe deficiency exposure on Fe redaction mechanism, photosynthetic gas exchange, chlorophyll fluorescence and antioxidant defense in two peach rootstock differing in Fe deficiency tolerance. Plant Physiol 163:176–185

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nikolic M, Kastori R (2000) Effect of bicarbonate and Fe supply on Fe nutrition of grapevine. J Plant Nutr 23:1619–1627

Pandolfini T, Gabbrielli R, Comparini C (1992) Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. Plant Cell Environ 15:719–725

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324–349

Pirasteh-Anosheh H, Emam Y, Rousta MJ, Ashraf M (2017) Salicylic acid induced salinity tolerance through manipulation of ion distribution rather than ion accumulation. J Plant Growth Regul 36:227–239

Raeisi S, Sharifi-Rad M, Quek SY, Shabanpour B, Sharifi-Rad J (2016) Evaluation of antioxidant and antimicrobial effects of shallot (*Allium ascalonicum* L.) fruit and ajwain (*Trachyspermum ammi* L. Sprague) seed extracts in semi-fried coated rainbow trout (*Oncorhynchus mykiss*) fillets for shelf-life extension. LWT Food Sci Technol 65:112–121